PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

WILSON, Kathryn, S.
Level 12
KPMG Centre, 85 Alexandra Street
Private Bag 3140
Hamilton
NOUVELLE-ZÉLANDE

Date of mailing (day/month/year)
10 July 2001 (10.07.01)

Applicant's or agent's file reference
41254PCX110

IMPORTANT NOTIFICATION

International application No.
PCT/NZ00/00159

International filing date (day/month/year)
17 August 2000 (17.08.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTE LIMITED
East Street
Ruakura Campus
Hamilton
New Zealand

State of Nationality: NZ
State of Residence: NZ
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

AGRESEARCH LIMITED
5th Floor Tower Block
Ruakura Research Centre
East Street
Hamilton, 2001
New Zealand

State of Nationality: NZ
State of Residence: NZ
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
A. Karkachi

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004140212

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

WILSON, Kathryn, S.
Level 12
KPMG Centre, 85 Alexandra Street
Private Bag 3140
Hamilton
NOUVELLE-ZÉLANDE

Date of mailing (day/month/year)
10 July 2001 (10.07.01)

Applicant's or agent's file reference
41254PCX110

IMPORTANT NOTIFICATION

International application No.
PCT/NZ00/00159

International filing date (day/month/year)
17 August 2000 (17.08.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTE LIMITED
East Street
Ruakura Campus
Hamilton
New Zealand

State of Nationality: NZ
State of Residence: NZ
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

AGRESEARCH LIMITED
5th Floor Tower Block
Ruakura Research Centre
East Street
Hamilton, 2001
New Zealand

State of Nationality: NZ
State of Residence: NZ
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
A. Karkachi

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004140212

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

WILSON, Kathryn, S.
Level 12
KPMG Centre, 85 Alexandra Street
Private Bag 3140
Hamilton
NOUVELLE-ZÉLANDE

Date of mailing (day/month/year)
27 April 2001 (27.04.01)

Applicant's or agent's file reference
41254PCX110

IMPORTANT NOTIFICATION

International application No.
PCT/NZ00/00159

International filing date (day/month/year)
17 August 2000 (17.08.00)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
LEWIS, Mardi, J.
65-67 Worcester Boulevard
P.O. Box 2201
Christchurch
New Zealand

State of Nationality:
State of Residence:
Telephone No.: 64 3 379 3664
Facsimile No.: 64 3 379 8220
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
WILSON, Kathryn, S.
Level 12
KPMG Centre, 85 Alexandra Street
Private Bag 3140
Hamilton
New Zealand

State of Nationality:
State of Residence:
Telephone No.: 64 7 957 5660
Facsimile No.: 64 7 957 5661
Teleprinter No.:

3. Further observations, if necessary:
The new agent's address on the Demand has been co nsidered as a change under Rule 92bis. In case of disagreement, the International Bureau should be notified immediately.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Claudio Borton

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference 41254/20jm	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/NZ00/00159**	International Filing Date *(day/month/year)* 17 August 2000	Priority Date *(day/month/year)* 17 August 1999
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] C12Q 1/34, 1/54		
Applicant NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTE LIMITED et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
9 March 2001	18 April 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **PHILIPPA WYRDEMAN** Telephone No. (02) 6283 2554

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ00/00159

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**1-23**	**YES**
	Claims	**None**	**NO**
Inventive step (IS)	Claims	**1-23**	**YES**
	Claims	**None**	**NO**
Industrial applicability (IA)	Claims	**1-23**	**YES**
	Claims	**None**	**NO**

2. Citations and explanations (Rule 70.7)

Novelty (N)

All the documents cited in the ISR were category A only. Therefore the claimed invention is not disclosed in any of these patent documents and hence all the claims are novel.

Inventive Step (IS)

The claimed invention is not obvious in the light of any of the cited documents nor disclosed in any obvious combination, nor would the claimed invention be obvious to a person skilled in the art in the light of common general knowledge by itself or in combination with any of these documents.

Industrial Applicabiiity (IA)

The claimed material is considered Industrially Applicable.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number
WO 01/12844 A1

(51) International Patent Classification[7]: C12Q 1/34, 1/54

(21) International Application Number: PCT/NZ00/00159

(22) International Filing Date: 17 August 2000 (17.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
337276 17 August 1999 (17.08.1999) NZ

(71) Applicants *(for all designated States except US)*: **NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTE LIMITED** [NZ/NZ]; East Street, Ruakura Campus, Hamilton (NZ). **NEW ZEALAND MEAT BOARD** [NZ/NZ]; 10 Brandon Street, Wellington (NZ).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WEST, John** [NZ/NZ]; 66 Nevada Road, Hamilton (NZ). **HART, Alan, Leedham** [NZ/NZ]; 29 St John's Avenue, Palmerston North (NZ). **YOUNG, Owen, Archibald** [NZ/NZ]; 4 Constance Street, Hamilton (NZ).

(74) Agents: **LEWIS, Mardi, J.** et al.; 65-67 Worcester Boulevard, P.O. Box 2201, Christchurch (NZ).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/12844 A1

(54) Title: RAPID METHOD FOR MEASURING COMPLEX CARBOHYDRATES IN MAMMALIAN TISSUE

(57) Abstract: The invention discloses a rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of: extracting a sample of tissue to be tested; forming a homogenous slurry of the sample with an aqueous solution; adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of glycogen in the slurry; and measuring the concentration of glucose in the slurry. The method can be conducted post-mortem to assay the concentration at the time of slaughter. The invention further discloses the measurement of lactate concentrations. The method of the invention can be conducted in thirty minutes or less.

TITLE: RAPID METHOD FOR MEASURING COMPLEX CARBOHYDRATES IN MAMMALIAN TISSUE

TECHNICAL FIELD

The present invention relates to a rapid method for measuring complex carbohydrates, particularly glycogen, in mammalian tissue. More particularly the present invention relates to the rapid measurement of glycogen in non-living mammalian tissue.

BACKGROUND ART

There are presently a number of methods of measuring complex carbohydrates, and particularly glycogen, in mammalian tissue. The discussion of these follows. The relevance of the measurement of glycogen includes, for example, the ability to use the results of glycogen measurement as a determination of the ultimate pH of meat. This in turn is a direct measure of many of the qualities of meat.

There are a number of known methods of measuring ultimate pH in meat: including use of liquid nitrogen in a freeze/thaw process and the use of a pH electrode for pH determination. There are a number of variations of this method, also. However the maintenance and use of liquid nitrogen in the quantities needed for the measurement on a continuous series of carcasses reveals hazards for the work environment. Also there is some doubt as to the accuracy and consistency of such measurement methods.

Methods of Measurement of Glycogen or Metabolites in Meat Samples

The iodine method: The principle of this method is that glycogen will react with a mixture of iodide, iodine and calcium chloride, forming an amber pigment in acid solution that has a linear absorption at least over a small, specified range. The glycogen is extracted from the meat with perchloric acid that is then filtered and centrifuged to recover a solution of glycogen which is reacted with the iodine. The extraction can also be by liquid nitrogen, potassium hydroxide, ethanol and ammonium chloride.

However, methods of extraction and then assay are time-consuming and employ aggressive chemical reagents.

Hydrolysis of glycogen with enzymes: The principle of this method is that glycogen hydrolyses to glucose, after which standard methods of measurement of free glucose may be used. The amyloglucosidase method of Dreiling et al (Meat Science, Vol 20, p. 167) is one such method, although other enzymes may be used. A muscle sample is homogenised with perchloric acid, and centrifuged, The supernatant, containing dissolved glycogen, is neutralised. Amyloglucosidase is added, converting the glycogen to glucose, for measurement. The first part of the method takes approximately 30 minutes at 37°C.

However there are some instances with the processing of meat in which a test of some 30 minutes or more is too long a time to wait for the test results, and some reagents are aggressive.

It is an object of the present invention to provide a rapid method for glycogen measurement in mammalian tissues, and in particular non-living tissue. It is a further object of the present

invention that in addition to the provision of a rapid accurate measurement of glycogen in tissue, the method also utilise mild or non-aggressive reagents.

Whilst the prior art and uses of glycogen measurement have been described with reference to a determination of the ultimate pH in meat, it will be appreciated that this method is not limited thereto. For example, low glycogen levels in the liver of bobby calves are an indicator of inadequate feeding before slaughter. Thus such rapid method of assessment of these levels could assist in ensuring good animal welfare.

It is an object of the present invention to address the foregoing problems or at least to provide the public with a useful choice.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

DISCLOSURE OF INVENTION

For the purposes of this specification, the term "rapid" is used to refer to times of less than 30 minutes and, more preferably, significantly less than 30 minutes

According to one aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of:

(a) extracting a sample of tissue to be tested;

(b) forming a homogenous slurry of the sample with an aqueous solution;

(c) adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of

glycogen in the slurry; and

(d) measuring the concentration of glucose in the slurry.

Advantageously, said complex carbohydrate is glycogen. Advantageously, said aqueous solvent is water. Optionally said solvent may include at least one agent intended to standardise ionic conditions and/or facilitate the steps of the above method.

Preferably the formation of the homogenous slurry is effected with a high or a low speed homogeniser, or with an ultrasonic apparatus.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (b) and (c) are performed simultaneously.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (c) and (d) are performed simultaneously.

Advantageously, the hydrolysing enzyme may be any enzyme, or combination of enzymes, capable of hydrolysis of glycogen to glucose.

According to a still further aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, in which said hydrolysing enzyme is selected from the group: amyloglucosidase; α-amylase; α-glucosidase, and a combination thereof.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above,

wherein the amyloglucosidase added in step (c) is in a form selected from: a powder; a liquid suspension; and a solution.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, in either embodiment, wherein said method further includes a step (e): measuring the concentration of lactate in the sample.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (d) and (e) are performed simultaneously.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (c), (d) and (e) are performed simultaneously.

The measurement of both metabolites gives a good post-mortem estimate of the concentration of glycogen present in tissue at the time of death, no matter when the measurement is made.

Advantageously, the method is conducted at approximately room temperature, although it may be conducted at a temperature in the range 0°C to 100°C.

Measurement of Glucose

There are a range of methods for measuring glucose. For the present invention the most useful are those adapted from known technologies to measure glucose in blood. These are

usually based on the generation of hydrogen peroxide in stoichiometric proportion to glucose, as catalysed by glucose oxidase.

Therefore, according to a still further aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, and in which glycogen levels are measured and in which said measurement of the concentration of glucose is achieved by the construction of sensors incorporating said hydrolysing enzyme and glucose oxidase.

Measurement of Lactate

There are a range of methods for measuring lactate, including the standard NADH-linked method and those based on the generation of hydrogen peroxide in stoichiometric proportion to lactate, as catalysed by lactate oxidase. This latter compound can be incorporated into any sensor which may already incorporate glucose oxidase.

BRIEF DESCRIPTION OF THE DRAWINGS

Further aspects of the present invention will become apparent from the following example, which is given by way of example only, and with reference to the accompanying drawings in which:

Figure 1 is a graph of glucose concentration and glucometer reading of a glucose sample in acetate buffer;

Figure 2 is a graph of the glucometer reading for glucose and concentration of glucose

added to meat slurry samples;

Figure 3 is a graph of the kinetics of glucose formation from glycogen in acetate buffer in the present of amyloglucosidase;

Figure 4 is a graph of glucometer readings and the concentration of glucose added to a meat/acetate buffer slurry;

Figure 5 is a graph of glucose value and glycogen added to a post rigor meat slurry, at 5 minutes incubation at 55 °C;

Figure 6 is a graph of muscle glycogen concentration in post slaughter samples, using the method of measurement of the present invention;

Figure 7 is a graph showing the kinetics of glycogen loss, pH fall and lactate increase in a bovine muscle sampled after slaughter; and

Figure 8 is a graph of the repeatability of glycogen determination by the method of the present invention.

BEST MODES FOR CARRYING OUT THE INVENTION

Chemicals, Equipment and Meat.

Amyloglucosidase from the fungus *Aspergillus niger*, in powder form, was added to meat slurries as a liquid suspension or solution. In suspension, 25 mg amyloglucosidase was dissolved in 5 ml of 3.2M ammonium sulphate and adjusted to pH 6.0 with ammonia. This particular solvent is known to be one in which the enzyme is stable. As an alternative, a clear solution can be obtained by the use of 25 mg of powder, dissolved in 5 ml of 0.2M sodium

acetate at a pH of 4.5. α-D-Glucose of a standard analytical grade was used.

Glucose measurement was made with an Esprit glucometer (Bayer). Test sensors used in the Esprit were from Bayer New Zealand Limited. These were used for one reading only then discarded.

The meat samples tested were obtained from the *longissimus lumborum* muscle of a beef carcass, obtained from a butcher. Pre-rigor muscle, variously *gluteus medius*, *semitendinosus* and *longissimus lumborum*, was obtained from an abattoir. These muscles were dissected from unstimulated carcasses approximately 25 minutes after slaughter and tested very shortly thereafter. The muscles were held at room temperature while measurements were made.

Glycogen Test Procedure

The test medium for all experiments was 0.2M sodium acetate buffer at a pH of 4.4 and at a temperature of 55°C. In the experiments the muscle or meat (in samples of accurately known weight, but approximately 1 g) was homogenised in 5 ml of buffer, with a high speed Polytron shearing head. This was usually set at 25,000 rpm. Alternatively a lower speed homogeniser may be used, if so desired. The homogeniser may be a stainless steel paddle-like blade rotating at 2000 rpm in a steel cup within an interior shaped like a standard domestic Waring blender.

After homogenisation the enzyme solution was added. The volume of this solution was usually 200 µl, containing 1 mg of amyloglucosidase. The mixture was briefly shaken, then held at 55°C.

Small aliquots, of approximately 20 µl, were withdrawn at intervals with disposable pipettes,

and spotted onto plastic film. The glucometer sensor sampled these drops and returned a meter reading for glucose concentration in 30 seconds.

As a control, tests were also carried out with a range of glucose and glycogen concentrations in an acetate buffer to which no meat or meat samples had been added.

Lactate Measurement

In one range of experiments lactate concentration was also measured. At various times after slaughter, a crude aliquot of the slurry, containing homogenised muscle and amyloglucosidase was centrifuged in a micro-centrifuge (at 10, 000 rpm for 30 seconds). The clear supernatant was recovered and analysed for lactate concentration by the NADH-linked method.

Results

The results from the glucometer were in mg of glucose/dl. These results are tabulated in Fig. 1 of the attached drawings. It is noted that the relation was linear but that the readings were approximately double the glucose concentration in fact present and did not pass through the origin.

The exact reason for the approximate doubling of the readings is not known. However it is understood that this might relate to the characteristics of glucose in blood. α-D-glucose as a laboratory chemical dissolved in acetate at a pH of 4.5 may require a different calibration from that in blood. However, the failure of the straight line to pass through the origin

suggests that the acetate medium affected the sensor performance.

Example 1

Various samples of meat (the samples being as described above) between 0.90 and 1.16 g were homogenised in the high speed homogeniser in 5 ml of acetate buffer containing up to 16 mg of added glucose. This addition rate translates to approximately 267 mg/dl, assuming the density of meat is about 1 g/ml.

As can be seen from Fig. 2 of the attached drawings the relationship, the relationship between added glucose and the average meter reading is linear. The positive value of detected glucose with zero added glucose may be explained by the small quantities of glucose left over from glycolysis in the meat samples.

Example 2

The method of Example 1 was repeated over a range of samples and concentrations in which various quantities of glycogen (between 0 - 14 mg) were added to 5 ml of acetate buffer and the reactions were started by amyloglucosidase addition.

The results are as set out in Fig. 3 of the attached drawings, in which can be seen the glucose values peaked and declined slightly. The reason for the decline is not understood but it is possible that this may result from a contaminating activity in the enzyme preparation or from isomerisation reactions of glucose liberated from glycogen.

The data tabulated in Fig. 3 suggest that at even the highest concentrations of glycogen,

around 40 activity units of amyloglucosidase, are sufficient to fully hydrolyse the glycogen within approximately 5 minutes.

Example 3

The experiment the results of which are tabulated in Fig. 3 was repeated in the presence of rigor meat. Meat samples ranged from between 0.97 - 1.07 g in six tests. The results are as set out in Fig. 4 of the attached drawings.

Individual data points were abstracted to plot glycogen added against meter readings for 5 minutes after the amyloglucosidase addition. The results of this are shown in Fig. 5. A quadratic equation was fitted, but the shape was close to a straight line.

Example 4

Samples of *gluteus medius* and a *semitendinosus* muscle were removed from pre-rigor meat, cut to samples in the size between 0.95 - 1.05 g, and homogenised in 5 ml of acetate buffer. This slurry was then treated with amyloglucosidase. The tests began 1.3 hours after slaughter, first with the *semitendinosus* muscle and at that time the muscle contained only 3.8 mg glycogen /g. By 2 hours the glycogen level declined to 1 mg/g.

In contrast, *gluteus medius* muscle sample contained 13.1 mg/g at 2.3 hours post slaughter, and the value declined steadily with time. The results are as shown in Fig. 6.

This experiment was repeated using *longissimus lumborum* muscle from another animal. This muscle is frequently used as an indicator of high pH condition. Lactate concentrations

were also measured.

The above tests as described were carried out and the results are as shown in Fig. 7 of the attached drawings. In this method the measurement of lactate was as per the standard NADH-linked method rather than the use of a sensor based on lactate oxidase.

Example 5

The robustness of the preferred embodiment of the test of the present invention was further determined by the following: meter readings were recorded in which triplicate aliquots of glycogen were added to three replicate rigor meat samples weighing 1.0 +/- 0.05 g. The slurry was treated in the same manner as described above for Example 2. The results of this are set out in Fig. 8 of the attached drawings.

Whilst the Examples given above to show the best method of performing the invention are all with reference to meat samples (beef) it would be appreciated by those skilled in the arts that other tissue samples may be equally treated in like manner to produce glycogen measurement as a result.

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto without departing from the scope thereof.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

CLAIMS

1. A rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of:

(a) extracting a sample of tissue to be tested;

(b) forming a homogenous slurry of the sample with an aqueous solution;

(c) adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of glycogen in the slurry; and

(d) measuring the concentration of glucose in the slurry.

2. A rapid method of measuring complex carbohydrates as claimed in claim 1 wherein said method is performed in less than 30 minutes.

3. A rapid method of measuring complex carbohydrates as claimed in either claim 1 or claim 2 wherein said complex carbohydrate is selected from glycogen, lactate and a combination of these.

4. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said aqueous solvent is water.

5. A rapid method of measuring complex carbohydrates as claimed in any one of the

preceding claims wherein said aqueous solvent includes at least one agent to standardise ionic conditions obtaining for the method.

6. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein the formation of the homogenous slurry is effected with apparatus selected from: a high speed homogeniser; a low speed homogeniser and an ultrasonic apparatus.

7. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said hydrolysing enzyme is selected from the group: amyloglucosidase; α-amylase; α-glucosidase, and a combination thereof.

8. A rapid method of measuring complex carbohydrates as claimed in claim 7 wherein said hydrolysing enzyme is amyloglucosidase which is in a form selected from: a powder; a liquid suspension; and a solution.

9. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said method further includes a step (e): measuring the concentration of lactate in the sample.

10. A rapid method of measuring complex carbohydrates as claimed in any one of the

preceding claims wherein steps (b) and (c) are performed simultaneously.

11. A rapid method of measuring complex carbohydrates as claimed in any one of claims 1 to 9 wherein steps (c) and (d) are performed simultaneously.

12. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein steps (d) and (e) are performed simultaneously.

13. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein steps (c) to (e) are performed simultaneously.

14. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein said measurement of lactate concentration is by the NADH-linked method.

15. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein said measurement of lactate concentration is based on the generation of hydrogen peroxide in stoichiometric proportion to lactate, as catalysed by lactate oxidase.

16. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said glycogen measurement is effected by use of sensors which

incorporate said hydrolysing enzyme and glucose oxidase.

17. A rapid method of measuring complex carbohydrates as claimed in claim 16 wherein said sensor further includes lactate oxidase.

18 A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said method is carried out at room temperature.

19. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said method is performed post-mortem, providing a measurement of concentrations of complex carbohydrates at the time of death.

20. A rapid method of measuring complex carbohydrates as claimed in claim 19 wherein said method is performed wherein said measurement is up to half an hour after slaughter.

21. Measurement of ultimate pH by use of the method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said tissue is meat.

22. Measurement of ultimate pH by use of the method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said tissue is muscle.

23. Measurement of ultimate pH by use of the method of measuring complex carbohydrates as claimed in claim 22 wherein said muscle is selected from: the *longissimus lumborum*; *gluteus medius; semitendinosus* and *longissimus lumborum* muscles.

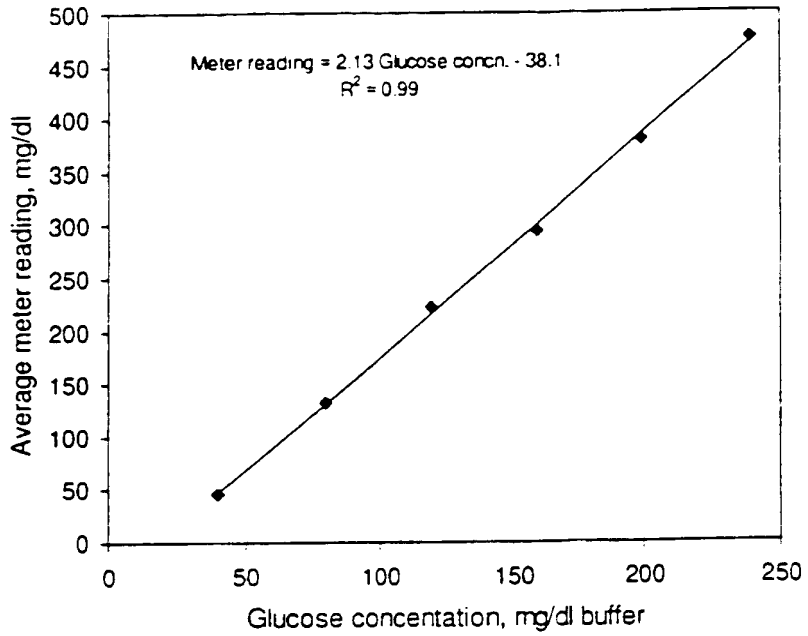

Figure 1

Relationship between the Esprit meter value for glucose and the concentration of α-D-glucose in acetate buffer at pH 4.5. Points are means of duplicates.

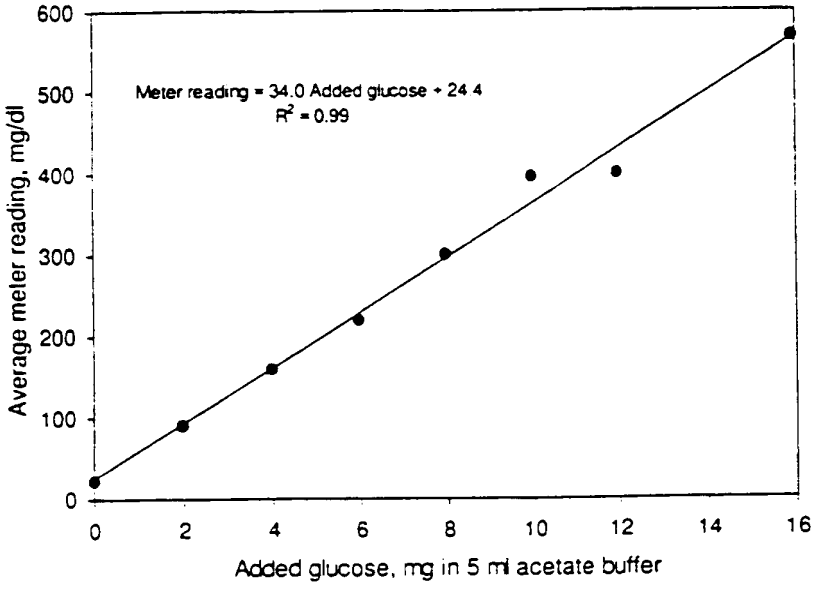

Figure 2

Relationship between the Esprit meter value for glucose and the concentration of α-D-glucose in a meat/acetate buffer slurry at pH 4.5. Points are means of duplicates.

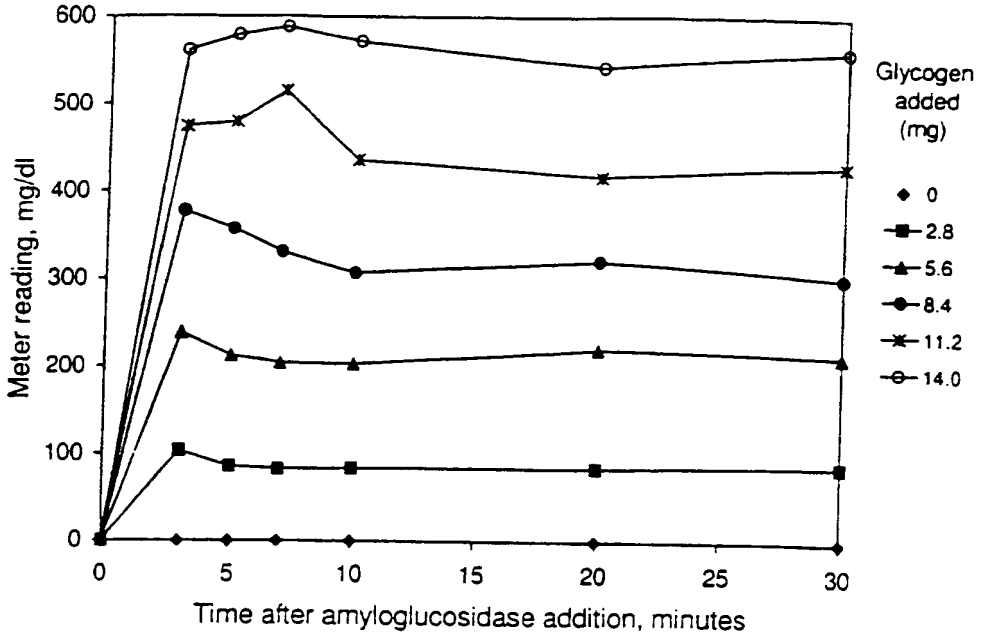

Figure 3

Kinetics of glucose formation from glycogen in acetate buffer in the presence of amyloglucosidase.

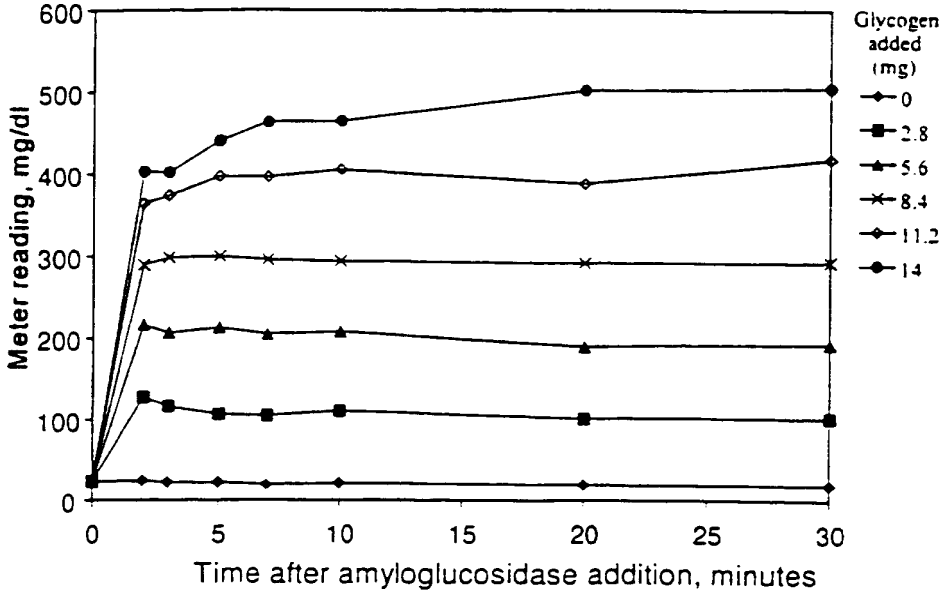

Figure 4

Kinetics of glucose formation from glycogen by amyloglucosidase in a meat slurry.

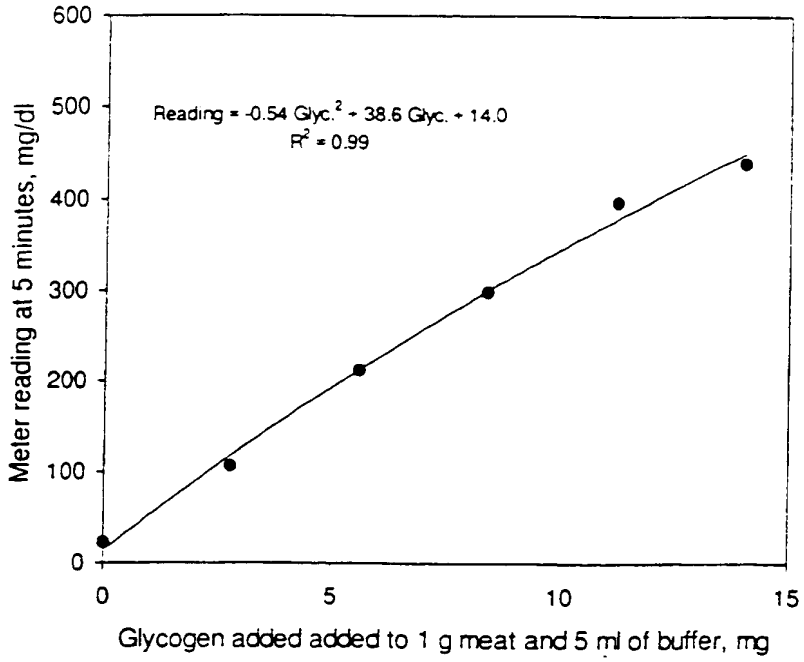

Figure 5

Relationship between the Esprit meter value for glucose and the quantity of glycogen in a post rigor meat slurry that also containing amyloglucosidase. Values were those recorded after 5 minutes incubation at 55°C. A quadratic equation was fitted to the data.

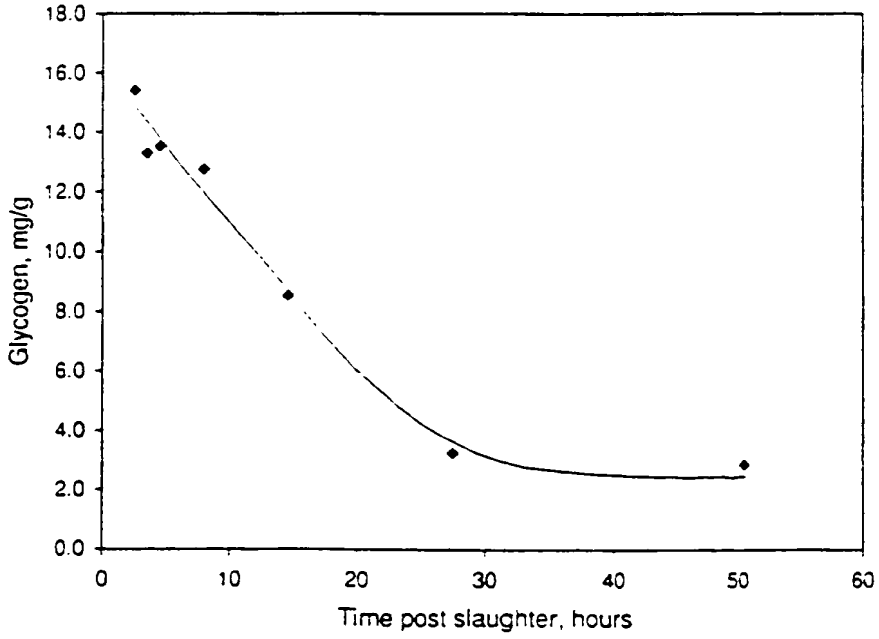

Figure 6

Kinetics of glycogen loss in *gluteus medius* as determined by the amyloglucosidase method.

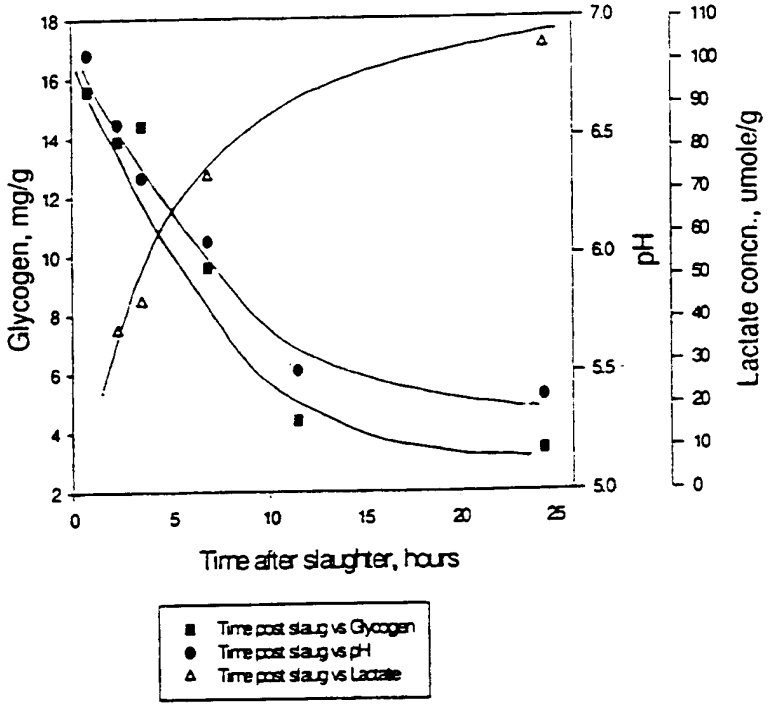

Figure 7

Kinetics of glycogen loss, pH fall and lactate increase in *longissumis lumborum* from an unstimulated carcass.

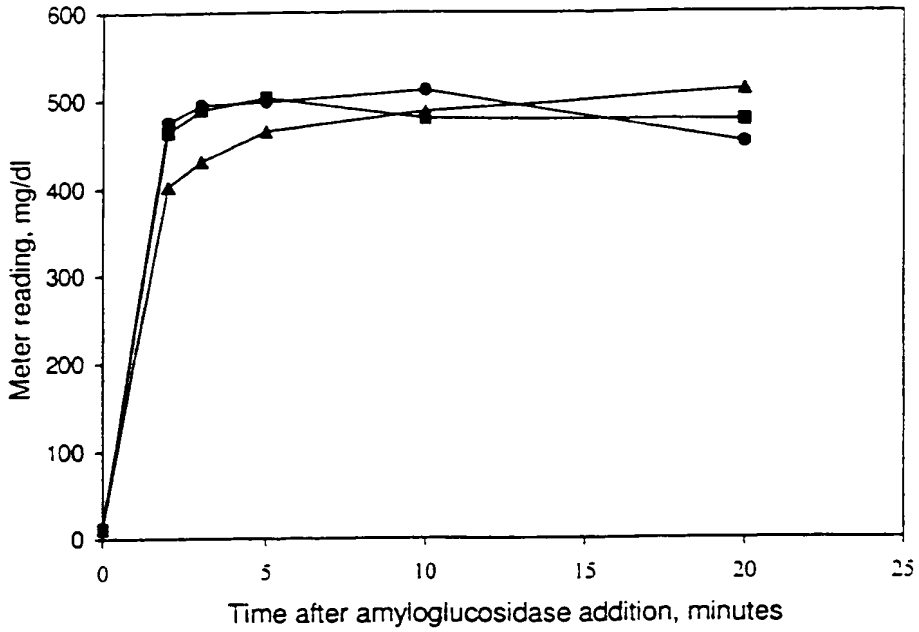

Figure 8

Repeatability of glycogen determination as glucose in the presence of rigor meat.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00159

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C12Q 1/34, 1/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE ELECTRONIC DATABASE BOX BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEE ELECTRONIC DATABASE BOX BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Chem Abs, Medline, WPIDS:
Keywords: tissue, meat, muscle, hydrolysis, hydrolyzing, enzyme, glucose, glycogen.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CALDER, P. C. and GEDDES, R. (1990) "Post Mortem Glycogenolysis is a Combination of Phosphorolysis and Hydrolysis" *Int. J. Biochem.* Vol. 22, No. 8, pp. 847-856. See abstract part 2 in particular.	

☐ Further documents are listed in the continuation of Box C ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 November 2000	14 NOV 2000
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	PHILIPPA WYRDEMAN Telephone No : (02) 6283 2554

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number
WO 01/12844 A1

(51) International Patent Classification[7]: C12Q 1/34, 1/54

(21) International Application Number: PCT/NZ00/00159

(22) International Filing Date: 17 August 2000 (17.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
337276 17 August 1999 (17.08.1999) NZ

(71) Applicants *(for all designated States except US)*: **NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTE LIMITED** [NZ/NZ]; East Street, Ruakura Campus, Hamilton (NZ). **NEW ZEALAND MEAT BOARD** [NZ/NZ]; 10 Brandon Street, Wellington (NZ).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WEST, John** [NZ/NZ]; 66 Nevada Road, Hamilton (NZ). **HART, Alan, Leedham** [NZ/NZ]; 29 St John's Avenue, Palmerston North (NZ). **YOUNG, Owen, Archibald** [NZ/NZ]; 4 Constance Street, Hamilton (NZ).

(74) Agents: **LEWIS, Mardi, J.** et al.; 65-67 Worcester Boulevard, P.O. Box 2201, Christchurch (NZ).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/12844 A1

(54) Title: RAPID METHOD FOR MEASURING COMPLEX CARBOHYDRATES IN MAMMALIAN TISSUE

(57) Abstract: The invention discloses a rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of: extracting a sample of tissue to be tested; forming a homogenous slurry of the sample with an aqueous solution; adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of glycogen in the slurry; and measuring the concentration of glucose in the slurry. The method can be conducted post-mortem to assay the concentration at the time of slaughter. The invention further discloses the measurement of lactate concentrations. The method of the invention can be conducted in thirty minutes or less.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00159

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C12Q 1/34, 1/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE ELECTRONIC DATABASE BOX BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEE ELECTRONIC DATABASE BOX BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Chem Abs, Medline, WPIDS:
Keywords: tissue, meat, muscle, hydrolysis, hydrolyzing, enzyme, glucose, glycogen.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CALDER, P. C. and GEDDES, R. (1990) "Post Mortem Glycogenolysis is a Combination of Phosphorolysis and Hydrolysis" *Int. J. Biochem.* Vol. 22, No. 8, pp. 847-856. See abstract part 2 in particular.	

☐ Further documents are listed in the continuation of Box C ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 November 2000	14 NOV 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer PHILIPPA WYRDEMAN Telephone No.: (02) 6283 2554